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THE TAXONOMY AND ECOLOGY OF THE HYDRIDAE (HYDROZOA)
OF ALBERTA AND THE NORTHWEST TERRITORIES

by

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A THESIS

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ABSTRACT

A study of the taxonomy, geographical distribution and ecology of the species of the Family Hydridae was conducted in the province of Alberta and a portion of the Northwest Territories in the summers of 1962 and 1963. Specimens were collected, identified, and if necessary cultured during this and the intervening winter period. Specimens from most localities were fixed in 70% alcohol. A method of isolation of nematocysts for viewing from fixed material is described.

A study of the effect of laboratory culturing on nematocyst length indicates a highly significant increase in the holotrich length of H. canadensis. A highly significant increase in holotrich length, a significant increase in stenotele length, and a significant decrease in atrich length of H. canadensis were also noted in relation to seasonal variation. A study of the effect of geographical variation indicates that neighboring localities show less difference in nematocyst length than do geographically remote localities.

A brief description and an account of the distribution and ecology are given for each of the species located in this study. For Alberta, these include two previously-unreported species, H. littoralis and Chlorohydra hadleyi, and three previously reported species, H. canadensis (H. pseudoligactis, New Synonymy), H. carnea and H. oligactis. For the N.W.T. new records of three species, H. canadensis, H. carnea and H. hymanae, are reported.

Each species appears to occupy a different habitat. The distribution of this habitat in turn determines the geographical distribution of each species.

The relationship of temperature and pH to sexual maturity is described in detail for H. carnea, H. canadensis and H. oligactis; fewer data are given for the other species studied.

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INTRODUCTION

The study of hydras has a long history. Leeuwenhoek in 1702 first remarked on the presence of these animals in a fresh water pond. The first detailed description and subsequent naming of these animals was left till 1744 when Abraham Trembley published his extensive work on the regenerative powers of these animals. Trembley distinguished three species but did not name them. Pallas in 1766 described Trembley's three species under the names Hydra viridissima, H. vulgaris and H. oligactis, and added a fourth, H. attenuata. Much further identification and reclassification continued after this initial work. Ewer (1948) in her review of the Hydridae lists 23 adequately described species, seven or eight of these being common to both the European and American continents. In addition to these he lists 16 incompletely described species from various portions of the world. Forrest (1963) in her key to the North American hydra records 15 species from this continent.

Previous study of the hydras of Alberta has been limited to the work done by Dr. W. Rowan. In 1930 Rowan reported the presence of Hydra canadensis n. sp. from lakes in the area of Edmonton, Alberta. This species has not been reported since from any locality. Specimens of hydras sent at that time by Rowan to L. H. Hyman in Chicago proved to be H. carnea Agassiz (Hyman, 1931). An element of doubt concerning the validity of canadensis thus arose. The situation

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was complicated by the omission of any data concerning the nematocysts in the description of this species, whereas in all modern accounts these structures are held to be of cardinal importance. Forrest (1963) states that "the description of H. canadensis (Rowan, 1930) is so incomplete, however, that without further information this hydra can not be surely identified". The present investigation was begun in 1961 by Mrs. P. C. Adshead and Dr. G. O. Mackie with the object of clarifying the taxonomic status of Rowan's H. canadensis, and of providing the missing details in the description. The results of their work are given by Adshead (1961). In the course of their study they found that, in addition to canadensis, two other species occurred in Alberta, H. oligactis and H. carnea. Another sample, obtained from Lake Wabamun in 1961, was provisionally designated as H. utahensis in their report. Because a number of taxonomically important characteristics were not observed, and no additional specimens have been found, judgement on the identity of this population is reserved. For reasons explained in this text this population was termed a variant population of H. hymanae in Adshead, Mackie and Paetkau (in press).

I continued this study in the summers of 1962 and 1963, extending it to include the geographical distribution and ecology of the species as well as their taxonomy. Most of the data were obtained in the summer of 1962. The summer of 1963 was devoted to obtaining additional data on distribution and to completion of the various experiments described below.



Figure 1. The method of searching for hydras in the littoral zone.



Figure 2. The method of searching for hydras in deeper water.



METHODS

Collection Methods

Sampling locations in Alberta were determined by the available transecting highways. Bodies of water were sampled at approximately every twenty miles in the region of these highways. In the Northwest Territories (hereafter abbreviated N.W.T.) most available bodies of water adjacent to the MacKenzie highway (to Yellowknife) were investigated.

Hydras were collected in two ways. In the littoral zone specimens were collected by examining plants and associated littoral debris in situ with the aid of a water telescope (Pennak, 1953) (Figure 1), and removing hydras thus located into a plastic bag for more extensive observation. If no hydras were located by this method, plants were placed in a bucket and these were examined for hydras within a twenty four hour period. In deeper water, plants were located from a canoe with the aid of the glass bottomed box (Figure 2). Plants thus located were brought to the surface with a long handled garden rake, and while still in the water were examined for the presence of hydras. Hydras located were placed in a bucket and examined within twenty four hours. A body of water was generally sampled at various arbitrarily selected locations taking into consideration difference in flora and in depth, as well as geographical or spatial differences. Ecological data were recorded for each collection site as indicated in a sample data sheet (Figure 3).

The temperature and pH of each body of water were taken at the first collection site, at a depth of approximately

Figure 3. Sample field data collecting sheet.

Hydra Distribution

I. Habitat.

Date: June 26, 1962.

Location: 3 miles west of Elkwater on Highway #48, south side of road.

Type of water: Slough, about 15-30 ft. wide, up to 6 ft. deep and clear in center. A valley slough.

Bottom type: Muddy

Depth: Hydras from one to four feet. Water to six feet, no plants there.

Abundance: About 50 per one foot stem of aquatic plant.

Associated fauna: Protozoa- Vorticella, Daphnia, Paramecium
Rotifera, mites, back swimmers, etc.

Associated flora: Potamogeton sp., Myriophyllum sp., and
Elodea sp.

Temp. air: 72° F. Water: 17°C.

Oxygen: 6.6 cc. per liter at 10:30 A.M.

P.H. 9.3

II. General characteristics:

Shape: no definite differentiation into stalk and body.
hypostome rounded

"A sketch was generally included."

Size: 8 to 10 mm. when captured, 9 to 12 mm. after warming up.

Color: Bright red and orange.

Tentacles:

Size to body length: 1 to 1.5 times as long

How held: Relatively erect, little droop, appeared

as a cup shaped flower.

"A sketch was generally included."

Number: 10 animals with six, three with seven.

Arrangement and form in fully contracted state, plus

order of budding. "A sketch was generally included."

III. Nematocysts:

"A separate page was devoted to the nematocysts. The following details were noted for each nematocyst type."

structure:	size:	relative abundance:
a free hand drawing	twenty meas-	in relation to
and camera lucida	urements were	other nematocyst
drawing.	taken.	types.

IV. Sex Organs:

"A separate page was devoted to the sex organs.

Details noted were:

State of sexuality: asexual or sexual
number of buds

Mon. or dioecious:

Testes: location on body, shape, number and size, complete with drawing.

Ovaries: location on body, shape, number and size, complete with drawing.

Theca: type, number and length of spines if present, complete with drawing.

two feet, or where this was not possible, from a bucket of water, soon after it was obtained. Temperatures were taken with a Students thermometer, pH with B.D.H. Universal Indicator solution.

Identification Methods

Identification of hydras is based on a number of morphological characteristics: the length of the column, number of tentacles, shape and size of testes and theca, and the length of nematocyst capsules. The characters generally used are indicated in a composite schematic drawing of a hydra (Figure 4). This study utilized all the characters generally used in hydra taxonomy except the order of the budding of the tentacles. This character is difficult to observe under field conditions and is not necessary for accurate identification of species of Hydridae.

General characteristics (as indicated in the data sheet, Figure 3) were noted in representative specimens from each collection site. These observations were verified and extended within twenty-four hours by examinations made with a binocular microscope. A total of approximately 300 specimens were identified to species. Examination and measurement of tentacular nematocysts was made in the field using a Wild M-20 microscope, the light being powered by a 6 volt battery. Nematocysts were measured at an approximate magnification of 1000X with an eyepiece micrometer, using phase contrast and bright-field microscopy.

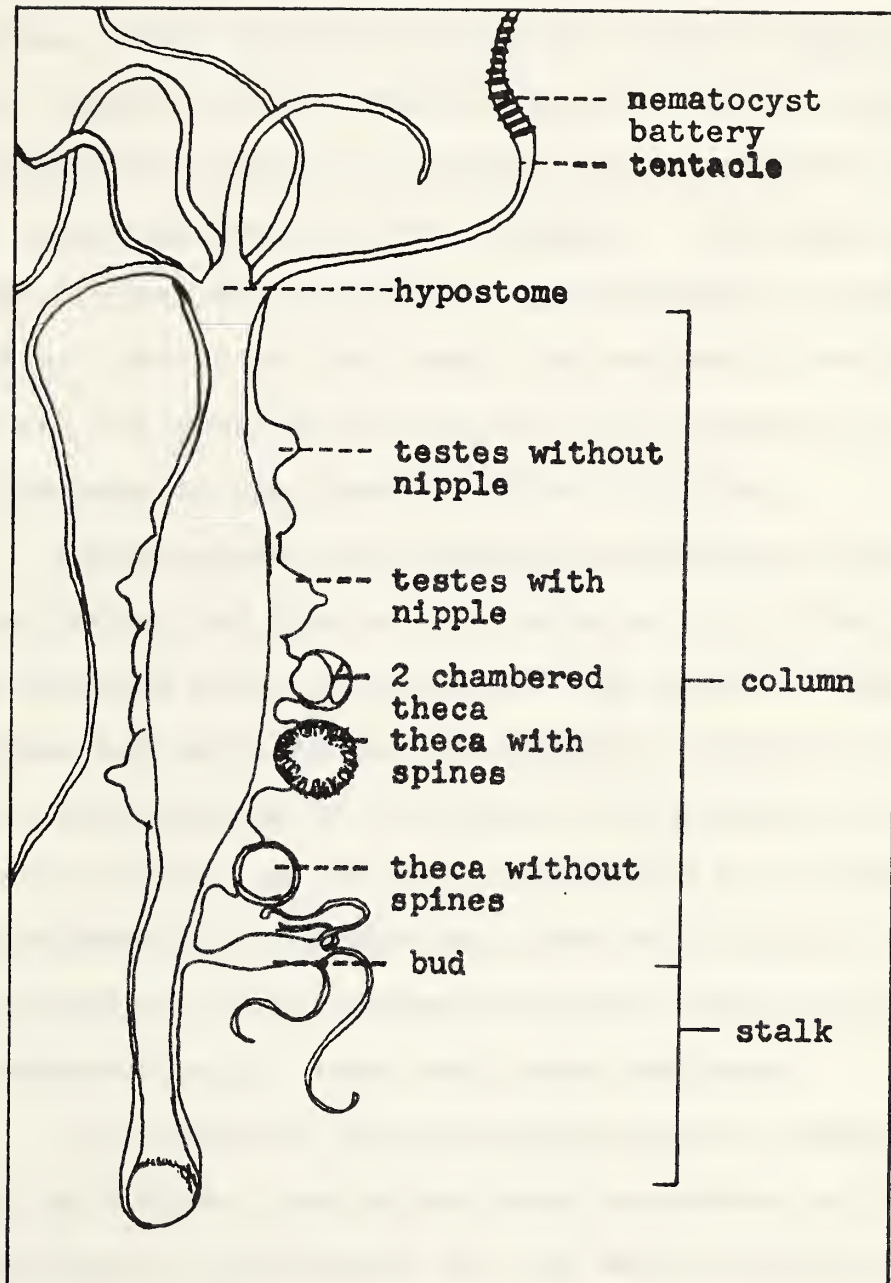


Figure 4. A composite, schematic drawing of a hydra.

Measurements of tentacular nematocysts only were used for identification. A statistical study by Itô (1949b) of Pelmatohydra robusta Itô indicated that the nematocysts on the tentacles were larger than those on the column. Semal-Van Gansen (1954) found this was also true for Hydra attenuata Pallas. Burnett (1959), using chemical methods, has shown that nematocysts migrate by normal growth processes from the growth region in hydra to the periphery - tentacles and peduncle. These nematocysts are structurally, though not chemically, mature as they reach the tentacular regions. Therefore, for consistent results, only tentacular nematocysts should be used in the identification of hydras.

Nematocysts were obtained by severing a tentacle from the animal and placing the tentacle on a slide where it was squashed under a coverslip. The overall length, general shape and variation in the internal coiling of the threads, particularly the coiling of the thread in the holotrichous isorhiza, are used as criteria for identification. The measurements for nematocysts from each locality are based on a minimum of twenty randomly-selected nematocysts of each type representing not less than three specimens.

The validity of these morphological characters as indicating distinct species has been determined on the basis of fertilization experiments for the North American forms, Hydra oligactis, H. pseudoligactis (a synonym of H. canadensis, see below) H. littoralis, H. hymanae, H. cauliculata, H. rutgersensis, and Chlorohydra hadleyi. H. carnea, H. americana

and H. minima have been tested less thoroughly, but no cross fertilization of these with other forms has been observed (Forrest 1963).

Fixation Methods

Isolation of the nematocysts from the fixed material was attempted using a pepsin digestion technique suggested by Mackie (1960). Modifications of this method were found to be necessary for the material under study. The method proposed for the isolation of hydra nematocysts from fixed material is as follows:

A complete, fixed animal is grasped by its peduncle with forceps, briefly dipped in distilled water (to remove excess fixative, thus avoiding subsequent inactivation through dispersal of the enzyme) and placed on a slide. Tentacles are then severed from the body, which is discarded. A drop of a 1 per cent solution of pepsin adjusted to a pH of 1.5 to 2.5 with 10 N HCL is placed on the tentacles. Digestion is allowed to proceed under the watchful eye of the observer. After approximately one minute the tissue under study begins to disintegrate and the nematocysts are released. At this point digestion is halted by neutralization with a drop of NH_4OH placed over the digestion mixture. A cover slip is placed over the material and the preparation is viewed in the same manner as "live" material.

The possibility that isolation of nematocysts was due solely to the acid used for pH control was tested. It was found that acid alone does produce some disintegration of the tissue, but that complete isolation is due to digestion by the enzyme.

No digestion was demonstrated for material fixed in Bouin's, Allen's B-15, 5 per cent formalin, or chromic acid. Digestion did occur in material fixed in absolute alcohol, 70 per cent alcohol or Carnoy's fixative. Cantarow and Schepartz (1960) state that pepsin is an endopeptidase which: 1) acts preferentially on linkages involving the amino group of tyrosine and of phenylalanine, and 2) has a pronounced effect on denatured proteins which possess free sulfhydryl groups. These statements indicate that this enzyme, like any enzyme, requires an activated site on the substrate so as to form the enzyme-substrate complex which is necessary for enzyme reactions. The reaction of formaldehyde is to link protein molecules into a net (Baker, 1960). It can be suggested that when the tissue was fixed in Bouin's, Allen's B-15, or 5 per cent formalin (all of which contain formaldehyde), the fixative had covered the active site for enzyme action, in this case probably free sulfhydryl groups. The formation of a net thus precluded pepsin digestion. The same type of enzyme inhibition could be suggested for chromic acid. Baker (1960) suggests that when proteins are precipitated by chromic acid a chemical combination occurs between the two. This chemical combination would probably bind or obscure any free sulfhydryl groups or other sites for formation of the enzyme-substrate complex.

Alcohol does not form a compound with protein, rather it abstracts water from it and denatures it irreversibly (Baker, 1960). This would suggest that fixation by alcohol

TABLE I

Data on the effect of fixation in 70% alcohol on stenotele length. Values given are means (in microns) of twenty measurements.

	Specimen No.	"Live"	Fixed	Difference
<u>H. carnea</u> no.	1.	15.6	12.0	3.6
	2.	14.8	11.7	3.1
	3.	15.2	12.0	3.2
	4.	15.3	12.0	3.3
<u>H. littoralis</u> no.	1.	16.7	13.2	3.5
	2.	16.3	13.2	3.1
	3.	15.6	13.3	2.3
	4.	15.3	12.6	2.7
<u>H. canadensis</u> no.	1.	14.6	11.5	3.1
	2.	12.9	10.4	2.5
	3.	13.6	11.3	2.3
	4.	14.0	11.3	2.7

TABLE I

Data on the effect of fixation in 70% alcohol on stenotele length. Values given are means (in microns) of twenty measurements.

	Specimen No.	"Live"	Fixed	Difference
<u>H. carnea</u> no.	1.	15.6	12.0	3.6
	2.	14.8	11.7	3.1
	3.	15.2	12.0	3.2
	4.	15.3	12.0	3.3
<u>H. littoralis</u> no.	1.	16.7	13.2	3.5
	2.	16.3	13.2	3.1
	3.	15.6	13.3	2.3
	4.	15.3	12.6	2.7
<u>H. canadensis</u> no.	1.	14.6	11.5	3.1
	2.	12.9	10.4	2.5
	3.	13.6	11.3	2.3
	4.	14.0	11.3	2.7

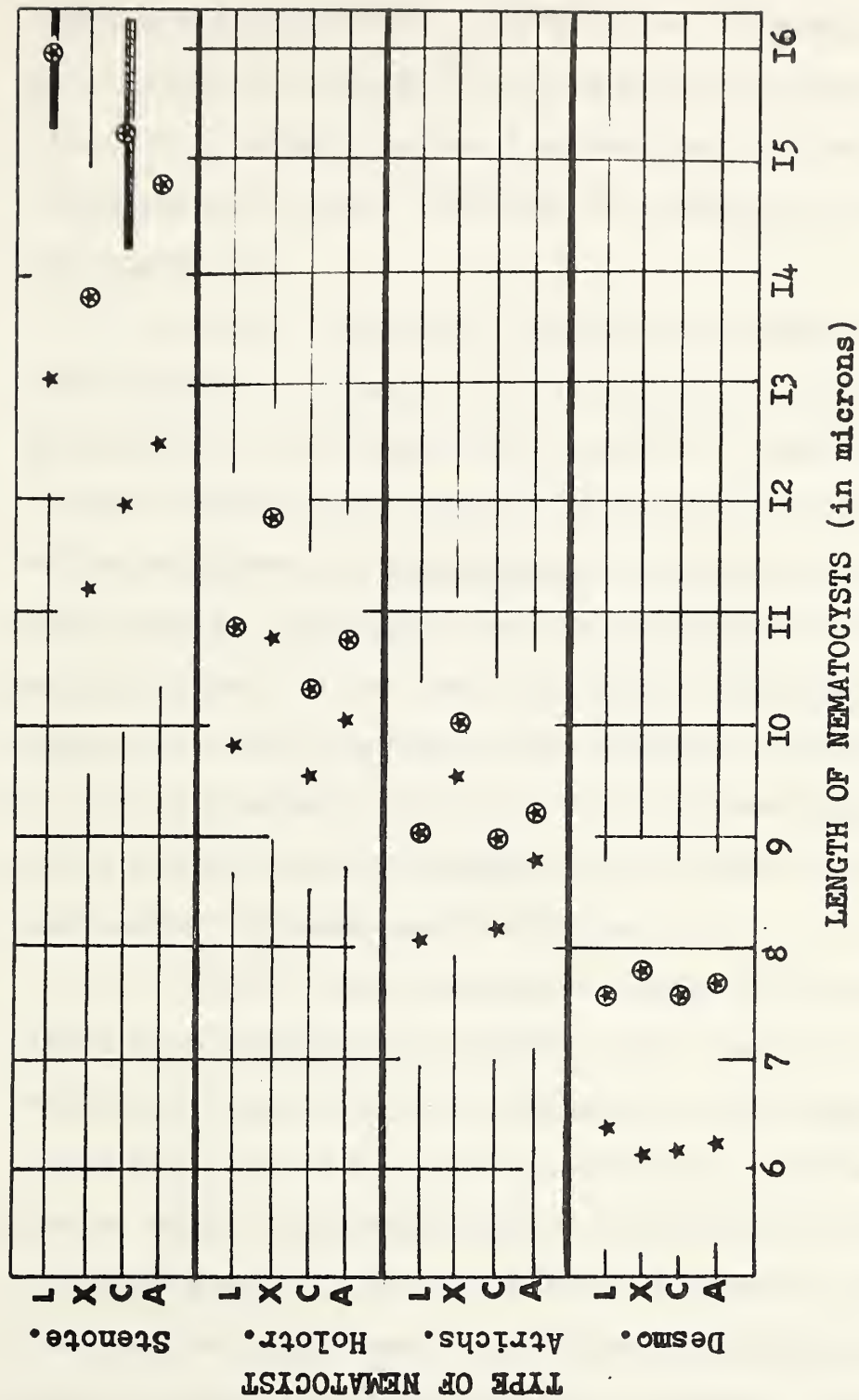


Figure 5. Data on effect of fixation in 70 per cent alcohol on nematocyst

length. ⊕ = "live" material, ★ = fixed material. L = *H. littoralis*,

X = *H. canadensis*, C = *H. carnea* (70% alcohol), A = *H. carnea*

(absolute alcohol).

(absolute, 70 per cent, or in Carnoy's mixture with acetic acid and chloroform) not only does not interfere with subsequent digestion by pepsin, but may aid it by exposing active sites. As a result isolated nematocysts can be prepared with tissue fixed in these solutions or probably in other comparable solutions.

Since 70 per cent alcohol is a satisfactory general field fixative a study of the alteration in size of nematocysts upon fixation in 70 per cent alcohol was undertaken. Specimens of three species were used for this study: H. carnea from Beddington Creek, H. canadensis from the Sturgeon River at St. Albert and H. littoralis from Little Cameron Lake. The results (Figure 5) are based on twenty measurements of each nematocyst type from each of four animals of each species. The relative effects of 70 per cent and absolute alcohol were also compared using H. carnea from the same locality and the same number of measurements (Figure 5).

Table I (for stenotele length of individual animals within each species) and Figure 5 (for the total sample, all nematocyst types within each species), show that the proportional change from "live" to fixed is relatively constant within species within nematocyst type. The greatest change in absolute size occurs in the stenoteles, followed in decreasing order by the desmonemes, holotrichs and atrichs. Material fixed in absolute alcohol appears to suffer less shrinkage than that fixed in 70 per cent alcohol in all nematocyst types except the desmonemes (Figure 5).

I had to use fixed material only once in this study, to determine the differences in nematocyst size between geographically isolated localities. The unit measurements taken from the fixed material were used, not the reconverted measurements suggested by Figure 5. I recommend that, in this group, fixed material be used for taxonomic purposes only when absolutely necessary. Even though external appearance, size and shape of nematocysts may be clearly outlined, some obliteration of internal structure does occur. Measurement of fixed material as compared to "live" material is also more tedious and time-consuming.

Culture Methods

It was desirable to maintain specimens in culture for study of certain features and to attempt to induce sexual maturity. In the field, specimens could be kept indefinitely in small, cheesecloth-covered bottles which were immersed in a bucket of pond or lake water.

In the laboratory hydras die in tap water (due to the copper, according to Chalkley and Park, 1947) or in distilled water (due to the lack of calcium, according to Loomis, 1954). Loomis and Lenhoff (1956) proposed a satisfactory culture medium and a useful culture technique. A modification of their method was used to maintain laboratory specimens in this study.

The modified culture medium consisted of 0.5 gm. CaCl_2 (dried), 22.5 cc. of Neptune stock solution (a pro-

prietary brand of artificial sea salt), and 10 liters of deionized water. This mixture was then siphoned into 1000 ml. finger bowls in which the hydras were maintained.

As suggested by the above authors the hydras were fed daily on the nauplius larvae of the brine shrimp Artemia salina, the dried and stable eggs of which may be purchased in large quantities and hatched (in a saturated salt solution) in daily batches of any desired size. Stagnation and contamination of the cultures was avoided by changing the culture medium within one-half hour and again about eight hours after feeding.

The identification of hydras involves the use of various mensural and meristic characters. Attempts were made to see whether these characters remained constant in spite of laboratory culturing.

General observations were attempted on variable characters such as the length of the column, column/tentacle length ratio, and tentacle number. The sample size proved too small for statistical analysis. Some data were, however, obtained from 4-6 month cultures of carnea and canadensis, which indicated that a general increase in the total length of the animal had occurred. A definite increase in the number of tentacles had also occurred in canadensis cultures (from an average of six to an average of eight). The possibility of change upon culturing in those important taxonomic characters, the nematocyst lengths, was also suggested and, as a result, was investigated.

Specimens of H. canadensis were obtained from the inlet canal of the power plant at Lake Wabamun on May 16, 1963 and specimens of H. carnea were collected from Elkwater slough on June 4, 1963. In each species six individual specimens were chosen at random from the population. A tentacle of each was severed, twenty of each type of nematocyst measured, and the remainder of the animal cultured individually for 50 (H. carnea) or 70 (H. canadensis) days. Two cultures of each species died during this test, probably due to high summer temperatures. At the end of the culture period two progeny from each of the remaining four cultures per species were selected. Twenty measurements per nematocyst type per animal were recorded for these progeny.

The use of progeny was thought to be valid in view of the work reported by Itô (1949a). He studied the morphological variation of the nematocysts of Pelmatohydra robusta and concluded with the statement, "In conclusion, there are recognized almost no significant differences between the size and the form of nematocysts in the bud and parent, and it may be noted that in the course of taxonomic study it is not necessary to take into consideration the biometrical characters of the nematocysts regarding the age of the asexually grown polyps".

The original and final nematocyst measurements were compared by the use of the nested analysis of variance test as outlined by Steel and Torrie (1960).

Only the holotrichs of canadensis showed a significant difference (an increase) between field and laboratory

TABLE II

Data on the effect of culturing on nematocyst length.

Values given are means in microns.

	Mean Length			
	. Before culturing 80 measure- ments	. After culturing 160 meas- urements	Variance ratio. F values	Signif icance
<u>H. canadensis</u>				
stenoteles	13.1	13.0	.1	n.s
holotrichs	11.7	12.3	11.2	H.S
atrichs	10.8	10.6	1.7	n.s
desmonemes	7.7	7.5	1.3	n.s
<u>H. carnea</u>				
stenoteles	16.0	16.0	-	n.s
holotrichs	10.3	10.4	.3	n.s
atrichs	9.1	9.2	.6	n.s
desmonemes	7.5	7.6	.7	n.s

n.s = not significant

S = significant, probability $< .05$ ($F > 4.96$)

H.S = highly significant, probability $< .01$

($F > 10.04$)

cultured animals (see Table II). Ito (1949b) showed that the penetrants (stenoteles) were the most variable while the glutinants (holotrichs) were the least variable. The measurements in this study (see description of each species) indicate the same relationship. Therefore, within the stenoteles, the variation within nematocysts as exposed by the large sample size and the method of analysis, is greater than the variation between the original and the laboratory cultured specimens. In the holotrichs, which exhibit less variation within the nematocyst type, the variation due to laboratory culture is better revealed. A highly significant increase was noted in the holotrichs of canadensis. The other two types of nematocysts do not appear to be greatly influenced by the environment.

H. carnea nematocysts did not exhibit any significant differences in this test.

The test described above revealed that differences in nematocyst lengths do occur in some cases. As a result only freshly caught material should be used in determining nematocyst lengths for taxonomic purposes. This suggestion was followed in this study.

TABLE III

Data on the effect of seasonal variation on the length of the nematocysts. Values given are means in microns of 80 measurements for H. canadensis and 60 measurements for H. carnea.

	. First measurement May 16	. Second measurement Sept. 27	. Variance ratio F values	Signif- icance
<u>H. canadensis</u>				
stenoteles	13.1	14.6	9.1	S.
holotrichs	11.7	12.7	28.9	H.S
atrichs	10.8	10.3	9.4	S
desmonemes	7.7	not measured	-	-

n.s = not significant

S = significant, probability $< .05$ ($F > 5.99$)

H.S = highly significant, probability $< .01$ ($F > 13.75$)

	. First measurement June 18	. Second measurement July 3	. Variance ratio F values	Signif- icance
<u>H. carnea</u>				
stenoteles	15.0	15.6	1.9	n.s
holotrichs	12.7	10.0	1.4	n.s
atrichs	10.2	9.5	-	n.s
desmonemes	7.6	7.6	-	n.s

n.s = not significant

S = significant, probability $< .05$ ($F > 7.71$)

H.S = highly significant, probability $< .01$ ($F > 21.20$)

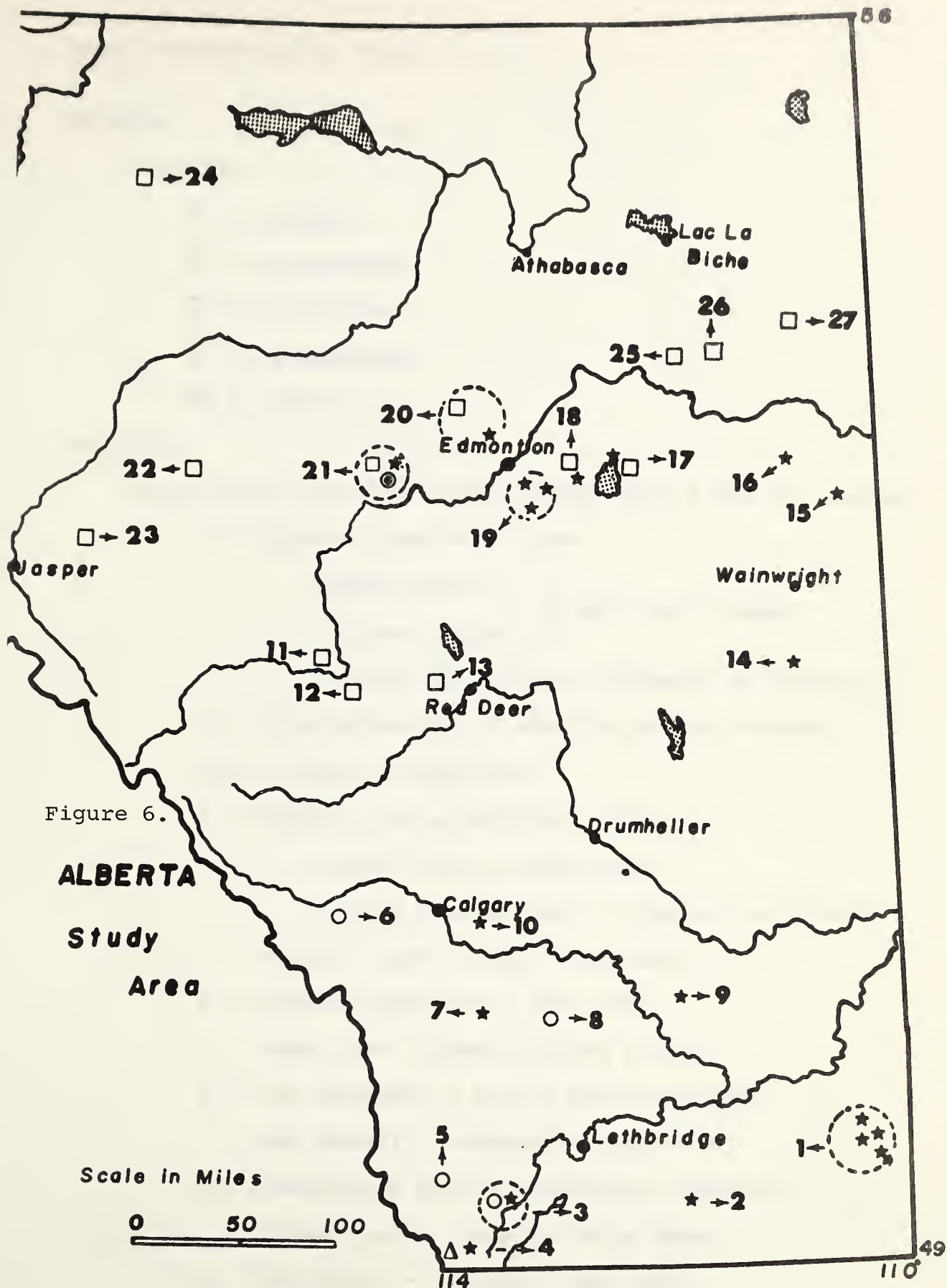
INTRASPECIFIC VARIATION IN NEMATOCYST LENGTHS

Recently various authors have commented on the possibility of environmentally-induced variations in the lengths of the capsules of the nematocysts amongst individuals or populations of the same species (Itô, 1951 and Semal-Van Gansen, 1954). These changes in lengths are important in the identification of hydras since such a great emphasis is placed on the range of absolute lengths. Two tests were attempted in this study (tests of seasonal variation and geographical variation) to see whether environmentally-induced changes also occurred in the two common local species, H. canadensis and H. carnea.

Seasonal Variation

Twenty measurements were made on each nematocyst type from each of four individuals of canadensis collected from Lake Wabamun power plant inlet canal on May 16, 1963 and Sept. 27, 1963. As Table III indicates, the holotrichs of canadensis showed a highly significant increase, the stentocytes a significant increase and the atrichs a significant decrease in length over the 134-day period.

Similar measurements were made on each of three individuals of carnea collected from Ellerslie slough June 18, 1963 and July 3, 1963. No significant differences were revealed in the H. carnea nematocysts.



Data incorporated in Figure 6.

Symbols:

Species:

- ★- H. carnea
- H. canadensis
- H. oligactis
- △- H. littoralis
- ⊙- C. hadleyi

Locality:

Only those localities where hydras were found are marked.

1. Elkwater Provincial Park

Reesor Lake	}	both small lakes
Spruce Coulee		

Slough three miles northwest of Elkwater.

2. Crow Indian Lake - shallow narrow reservoir

3. St. Mary's Reservoir -

4. Waterton Lakes National Park -

Linnet Lake - small pond

Little Cameron Lake - glacier fed stream

5. Beauvais Lake - small deep lake

6. Barrier Reservoir - deep lake

7. Frank Lake - large shallow slough

8. Lake McGregor - a long deep reservoir

9. Lake Newell - irrigation reservoir

10. Chestermere Lake - irrigation reservoir

11. Crimson Lake - moderate size lake

12. Twin Lakes - moderate size lake

REGULAR ARTICLES

1211-1224

1225-1238

1239-1252

1253-1266

REGULAR ARTICLES (continued)

1267-1280

1281-1294

1295-1308

1309-1322

1323-1336

1337-1350

1351-1364

1365-1378

1379-1392

1393-1406

1407-1420

1421-1434

1435-1448

1449-1462

1463-1476

1477-1490

1491-1504

1505-1518

1519-1532

1533-1546

1547-1560

1561-1574

1575-1588

1589-1602

1603-1616

1617-1630

Data incorporated in Figure 6 (continued):

13. Sylvan Lake - moderate size lake
14. Ribstone Creek -
15. Kitscoty slough
16. Slough 5 miles south of Sask. River
17. Beaverhill Lake - moderate size lake
18. Hastings Lake - moderate size lake
19. Ellerslie sloughs
Big Island Lake - shallow large sloughs
20. Sandy Lake -
Devils Lake
21. Wabamun Lake - large lake
22. Chip Lake - large lake
23. Talbot Lake - large lake
24. Sturgeon Lake
25. Cache Lake -
26. Upper Mann Lake - a large lake
27. Moose Lake - a large lake





Data incorporated in Figure 7.

Symbols:

Species:

★ = H. carnea

□ = H. canadensis

* = H. hymanae

Locality:

Only those localities where hydras were found are marked.

1. Enterprise to Kakisa River.

Mile 5.5 - a roadside dugout.

Mile 10.3 - a small, 3 to 4 ft. wide, creek.

Mile 23 - a substantial creek.

Mile 33 - a creek.

Mile 44.7 - 1/4 mile square shallow "lake".

Mile 50.2 - a clear water creek.

2. Kakisa River to Fort Providence.

1 mile west of Mile 66 - shallow "lake".

10 miles west of Mile 66 - a creek.

Great Slave Lake highway: Fort Providence
to Rae.

3. Mile 35 - large muskeg slough.

4. Mile 40 - a slow moving portion of Caen lake.

5. Mile 75 - portion of a large network of sloughs.

6. Rae to Yellowknife.

1 mile south of Frank Channel - hydras found
in lake inlet.

5 miles south of Stagg River.

Data incorporated in Figure 7 (continued):

6. Stagg River - small river.

25 miles west of Yellowknife - large flowing stream.

6 miles south of Yellowknife - small stagnant pool.

Geographical Variation

This study was undertaken to ascertain whether intra-specific differences occur between geographically isolated localities. Material gathered in the summer of 1962 and fixed in 70 per cent ethyl alcohol was used for this study. Nematocysts were isolated by the pepsin digestion method outlined above.

Specimens of H. canadensis from two Alberta localities, Crimson Lake west of Red Deer ; (no. 11 on Figure 6) and Moose Lake, southeast of Lac La Biche (no. 27 on Figure 6), and two localities in the Northwest Territories between Rae and Yellowknife (both at no. 6 on Figure 7) were compared. Three specimens from each locality were selected and twenty measurements made on each of two of the nematocyst types.

The results (Table IV) revealed no significant differences in stenotele lengths between the four localities but a highly significant difference in holotrich lengths. The holotrichs from the two Alberta localities did not differ, nor did the holotrichs from the two N.W.T. localities, but the Alberta forms were highly significantly larger than the forms obtained in the N.W.T.

Specimens of carnea from three localities, a slough near Elkwater in southeastern Alberta (no. 1 on the distribution map, Figure 6), Crow Indian Reservoir in southern Alberta (no. 2 on Figure 6), and a slough near Kakisa Lake, N.W.T. (no. 2 on Figure 7), were chosen for comparison. Three specimens from each locality were selected and twenty measurements made on each of two of the nematocyst types.

TABLE IV

Data on geographical variation in nematocyst length, H. canadensis. Values given are means (in microns) of 60 measurements taken from 70% alcohol fixed material.

	. Crimson Lake	. Moose Lake	. Yellow- knife	. Stagg river
stenoteles	13.0	12.7	13.2	13.0
holotrichs	11.8	11.9	10.3	10.3

(b) Results of the analysis of variance showing
F values.

	. Total	. Crimson Lake Moose Lake	. Y'knife Stagg R.	. Alberta N.W.T.
stenoteles	1.4 n.s	--	--	--
holotrichs	60.8 H.S	.6 n.s	.02 n.s	218.8 H.S

	<u>Total</u>	<u>Between lakes</u>
n.s = not significant		
S = significant, probability < .05	(F > 4.07)	(F > 7.71)
H.S = highly significant, probability < .01	(F > 7.59)	(F > 21.20)

A comparison of the holotrichs showed a significant difference between either Alberta form and the N.W.T. form but no significant difference between the two adjacent Alberta forms (Table V). Stenoteles of the specimens from the slough near Elkwater were found to be significantly different from both of the other two localities. Geographically remote localities showed a greater difference than the geographically neighboring localities. However, when stenoteles of the specimens from Crow Indian Reservoir were compared with the other two samples it was found that a significant difference existed between the neighboring localities, but no significant difference could be shown for the geographically remote localities.

Discussion and Conclusion

Itô (1951) reported a seasonal variation study of similar nature using Pelmatohydra robusta. In this study he noted the lengths of holotrichs and stenoteles of hydras collected in May, July, August, October and December from a pond in Sendai, Japan. He found that the size changes were insignificant for holotrichs but were significant for stenoteles. The size became smaller from spring into summer and larger from autumn to winter. My results for canadensis agreed with Itô's in that stenoteles showed a significant difference, however, holotrichs also showed a difference (highly significant) as did the atrichs. Itô did not measure the atrichs.

TABLE V

Data on geographical variation in nematocyst length, H. carnea. Values given are means (in microns) of 60 measurements taken from 70% alcohol fixed material.

	. Elkwater slough	. Crow Indian Reservoir	. N.W.T.
stenoteles	13.0	12.7	12.3
holotrichs	9.3	9.0	10.5

(b) Results of the analysis of variance showing F values.

	. Total	. Elkwater Crow Indian	. Elkwater N.W.T.	. Crow Indian N.W.T.
stenoteles	15.1 H.S	10.8 S	20.8 S	6.1 n.s
holotrichs	8.5 S	2.2 n.s	11.3 S	18.4 S

	<u>Total</u>	<u>Between lakes</u>
n.s = not significant		
S = significant, probability < .05	(F > 5.14)	(F > 7.71)
H.S = highly significant, probability < .01	(F > 10.92)	(F > 21.21)

The short period (15 days) allowed for producing any seasonal variation in H. carnea might be questioned. This was, however, the natural duration of the species in the slough studied.

Concerning geographical variation in nematocyst lengths, Itô (1951) found no significant difference in glutinant (holotrich) lengths between specimens taken from geographically adjacent localities. He did initially find significant difference in penetrant (stenotele) lengths. However, the significant difference in length of the penetrants disappeared after four months of culturing (Itô does not use the Loomis and Lenhoff method of culturing). Thus he concludes that "variations of the shapes and sizes of the nematocysts seem to be originally insignificant between these localities".

The results obtained from a study of canadensis agree with Itô in that no significant difference in stenotele or holotrich lengths could be shown for geographically adjacent localities. H. carnea did exhibit a significant difference in stenotele lengths if one considers the adjacent localities of Elkwater/Crow Indian. The stenotele sample from Crow Indian Reservoir, however, did not show any significant difference when compared with a geographically isolated locality - Kakisa Lake. Therefore, the importance of this stenotele sample must be viewed with caution.

Further study by Itô on three remote localities showed a significant variation on both stenoteles and holotrichs

between these isolated localities. The variations remained unchanged in spite of laboratory culturing for ten months. Semal-Van Gansen (1954) also demonstrated differences in tentacular nematocysts between two geographically isolated populations of H. attenuata Pallas.

If one considers only the stenoteles it appears as if our results are not in agreement with those reported above. Considering canadensis the method of analysis and large sample size once more illustrates that the variation within stenoteles is so great that it obliterates variation between samples. H. carnea did exhibit less variation between geographically adjacent localities as compared to isolated localities if one considers only the Elkwater/Kakisa Lake relationship. The difficulty in the Crow Indian/Kakisa Lake comparison has already been discussed. The results obtained from a study of the holotrichs did agree, in both species, with those reported by Itô and Semal-Van Gansen. The geographically isolated localities showed significant differences in nematocyst lengths.

Itô also reported a correlation between latitude and size in his study. He found that the farther north the locality (in Japan) the larger the size of both stenoteles and holotrichs. No trend of this nature could be demonstrated in this study.

The results of the above tests (effect of culturing, seasonal variation and geographical variation) all revealed a certain amount of intraspecific variation. Causal factors postulated for these differences may involve the different

functions of the nematocysts and may be related to the associated food. The stenoteles are used for piercing the prey, the holotrichs are used in defence, and the atrichs are thought to be of some use in locomotion. Therefore, a difference in food due to differences in culturing (hydras were fed a steady diet of Artemia salina larvae), seasonal variation in the natural fauna or geographical variation in the fauna may be the factor affecting the change in nematocyst lengths. Whatever the determining factor is, intraspecific variation in nematocyst length does occur and must be taken into consideration in the identification of hydras.

These tests once again show the importance of stating the conditions under which a specimen is obtained and observed. This is particularly important in taxonomy but should be remembered by non-taxonomists who use these animals for other purposes, as, for example, enzyme studies, growth studies, histochemical studies of the nematocysts, etc. (see Lenhoff and Loomis, 1961).

THE GENERA AND SPECIES OF HYDRIDAE OF ALBERTA
AND A PORTION OF THE NORTHWEST TERRITORIES

Genus Hydra Linnaeus

The fresh water hydroids included in this group are identified as being solitary polyps with tentacles, without a medusa stage in the life history, and without the zoochlorellae (green algae) in the gastrodermis.

Hydra carnea L. Agassiz, 1850

Description

H. carnea is a relatively small hydra with a slender column and typically without a stalk. However, a stalk was noted in some specimens which had recently fed. Column length does not exceed 10 mm. in freshly collected specimens; after a period of laboratory culture, however, some individuals attained lengths up to 16 mm. Hyman (1931) records 10 mm. as the maximum length in her material. The tentacles number five to eight, usually six. They measure 1.5 to 3 times the column length. A more complete description can be found in Hyman (1931).

The following range of measurements of the nematocysts was obtained in this study.

stenoteles	12.5 - 20.0 μ
holotrichs	9.0 - 12.5 μ
atrichs	7.5 - 11.5 μ
desmonemes	6.0 - 9.0 μ

The rest of the measurements are in between these extremes in an apparently normal distribution pattern. Adshead (1961)

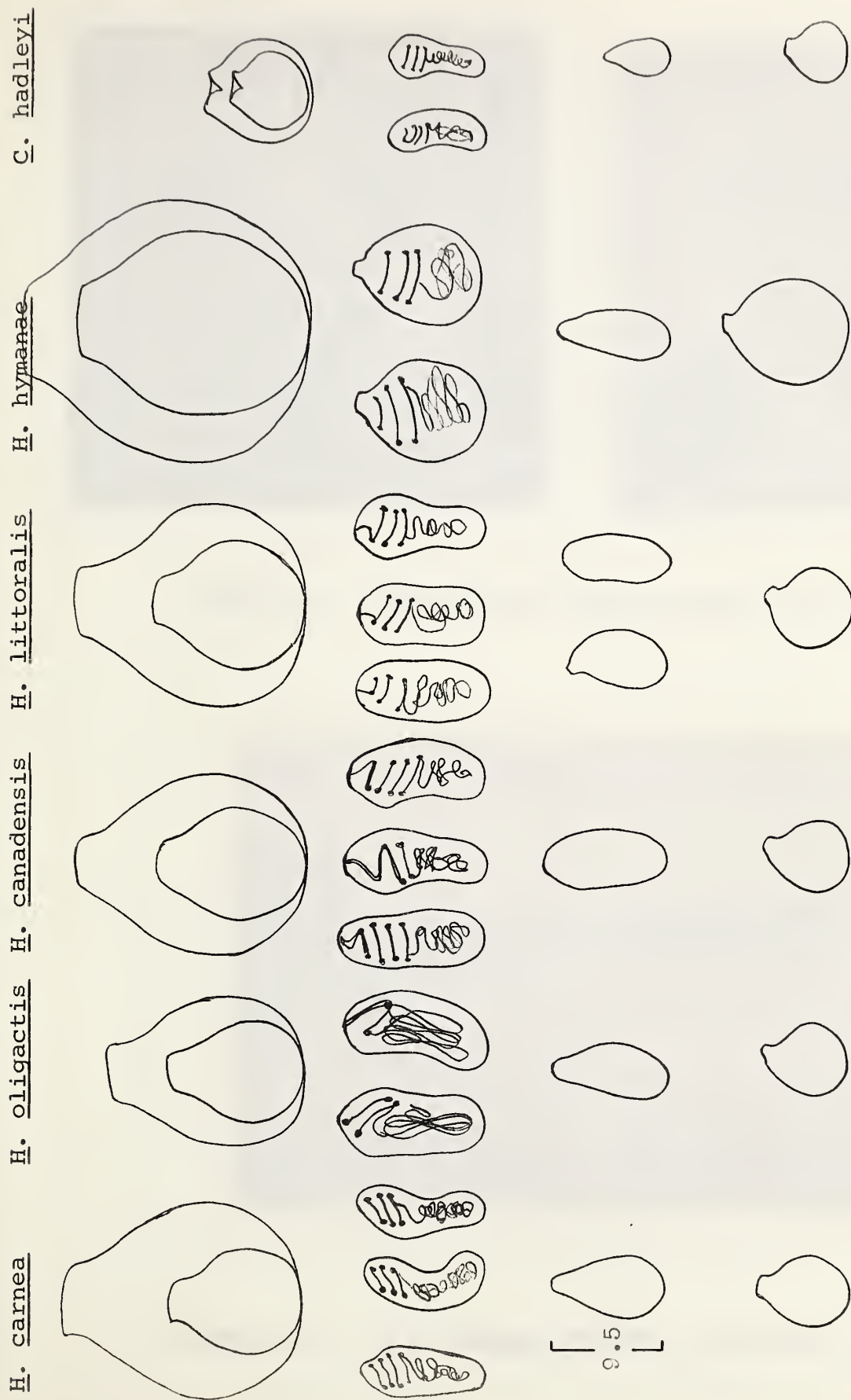


Figure 8. Nematocysts of the hydroids from Alberta and the N.W.T. drawn to the same scale. Top row, stenoteles, showing smallest and largest sizes found; second row, holotrichs (mean sizes), showing variation in internal and external structure; third row, atrichs (mean sizes); fourth row, desmonemes (mean sizes).



Figure 9. The spherical embryotheca of H. carnea.



Figure 10. A H. carnea habitat, Ellerslie slough.

reported a minimum stenotele size of 10.5μ from some specimens obtained in Alberta. The shapes of these nematocysts are indicated in Figure 8. The holotrichs are oval, pointed at the anterior end and frequently kidney or slipper-shaped, a fact not mentioned in Hyman's description.

This species is hermaphroditic, with marked protandry, as stated by Hyman. The testes have nipples. The embryonic theca is spherical with short, thick spines (Figure 9).

Distribution

Hyman recorded this species from localities throughout the eastern and central U.S.A. and, on the basis of Rowan's specimens, from lakes near Edmonton, Alberta (Beaverhill and Hastings).

H. carnea was found to be the common hydra of southeastern Alberta. It was located in a total of 20 localities in Alberta (Figure 6) and 4 localities in the N.W.T. (Figure 7). The Alberta locations are concentrated in the south-east portion of the province within the area bordered by Cypress Hills in the south-east, St. Mary's Reservoir in the south-west, Edmonton in the north-west and Lloydminster in the north-east. Within the area of the N.W.T. studied, (the Mackenzie highway to Yellowknife) carnea appears to be limited to the area south of the Mackenzie River. The discontinuous distribution of this species in this study seems to be related to its habitat preferences as described below.

Ecology

This species occurs most frequently in shallow,

TABLE VI

Data on the temperature and pH of H. carnea localities visited in June-July and Sept. 1962.

Location	Date	Temp.	pH	Species of <u>Hydra</u> present	Date	Temp.	pH	Species of <u>Hydra</u> present
Lake Newell no. 9	June 17	23	9	<u>H. carnea</u>	Sept. 4	17.5	9	none
Spruce Coulee Lake no. 1	22	18	8	<u>H. carnea</u>	5	8	8	none
Reesor Lake no. 1	-	18	8	<u>H. carnea</u>	-	-	-	-
Elkwater Creek no. 1	-	19	7.3	<u>H. carnea</u>	-	-	-	-
Elkwater slough no. 1	27	17	9.3	<u>H. carnea</u>	5	10	10	none
Crow Indian Lake no. 2	July 3	15	7.3	<u>H. carnea</u>	5	17.5	11	none
St. Mary reservoir no. 3	9	15	7.2	<u>H. carnea</u> <u>H. oligactis</u> (both rare)	5	16	7.5	<u>H. oligactis</u>
Linnet Lake no. 4	11	18	7.3	<u>H. carnea</u>	6	14	7.5	<u>H. carnea</u>
Frank Lake no. 7	14	16	8	<u>H. carnea</u>	8	15	9.5	none
Chestermere Lake no. 10	16	18	8	<u>H. carnea</u>	-	-	-	-

muddy-bottomed sloughs or ponds. It is rarely found in larger, cleaner bodies of water; when it is discovered in the latter, it is invariably located in a shallow portion overgrown with weeds and with a muddy bottom.

Hydra carnea utilizes various aquatic plants as substrates for attachment. It was often discovered on Typha sp., generally on the inner surface of a previous year's stalk or leaf. Substrate preferences following Typha sp. seem to be Potamogeton sp., Myriophyllum sp., and Elodea sp. in that order. However, where hydras were in abundance they could be obtained on all available objects: twigs, stones, snails and even caddis fly cases.

A field survey of the southeastern portion of Alberta, where carnea is found, was first made in the latter part of June and July of 1962. This survey yielded carnea from ten locations, eight of which were in the pH range of 7.3 to 8 (Table VI). Nine of these locations yielded asexual carnea while the location with the highest pH (9.3) produced sexual carnea. Further data of the effect of pH on H. carnea was obtained when certain localities were revisited in September 1962. At this time no hydras were obtained in certain locations in which carnea had been found in June and July. A general increase in pH had occurred in these lakes with the average pH being 9.5 (Table VI).

It appeared that the high pH might have been the cause of the disappearance of hydras from these locations. To test this a small slough (Figure 10) four miles directly east of Ellerslie, was chosen as a typical H. carnea habitat.

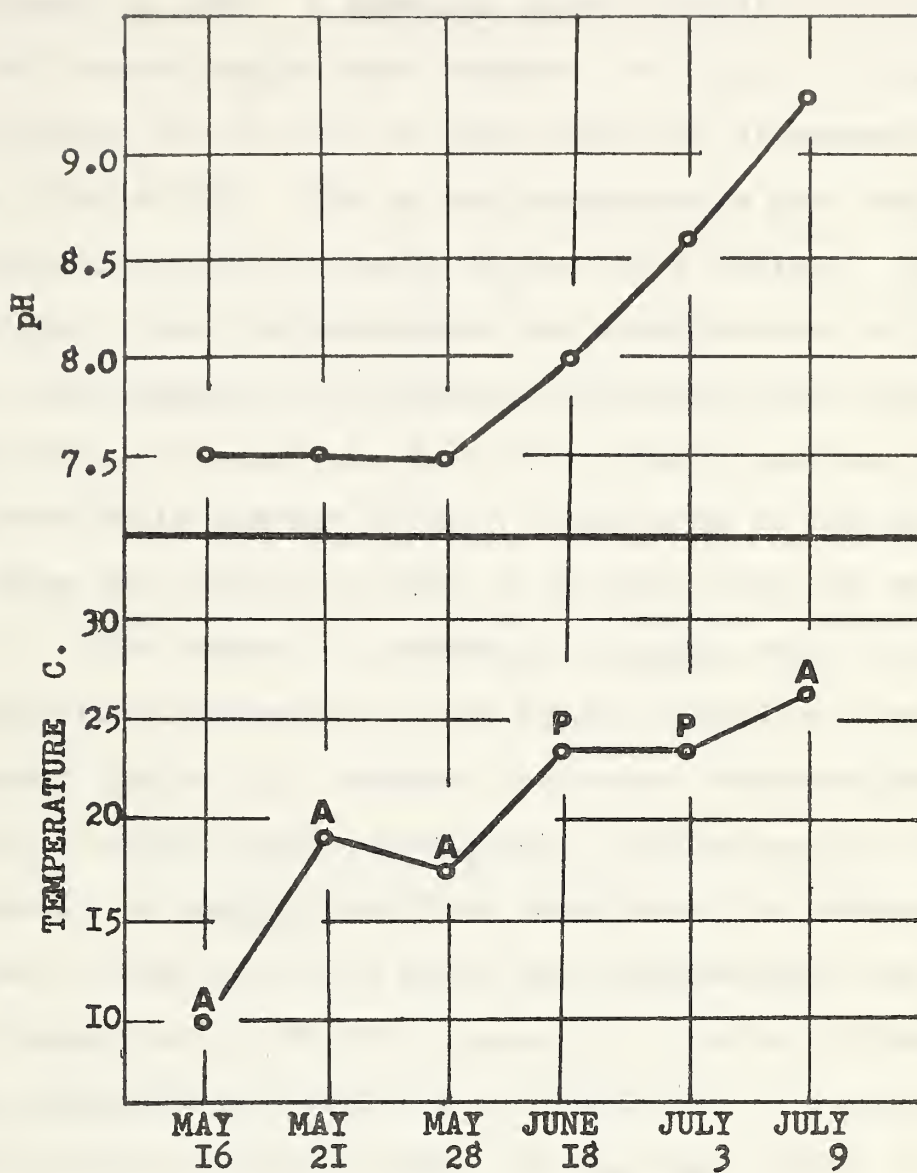


Figure 11. The seasonal variation in pH and temperature as related to H. carnea existence.

A = H. carnea absent

P = H. carnea present

Hydrogen ion concentrations, temperature and the presence or absence of hydras were recorded periodically during the spring and summer of 1963. A chemical water analysis was taken on May 29, 1963 before hydras were present, on July 4, 1963 when hydras were present and on July 10 just after the disappearance of hydras (Table VII). The pH and temperatures were recorded at the exact location and depth of the hydra habitat. The pH, the temperature, and the appearance and disappearance of carnea in this slough appear to be directly correlated (see Figure 11). Hydra were not noted till June 18 at which time the pH was 8. They were still present on July 3 with a pH of 8.6 but were absent from the locality on July 9 at which time the pH was 9.3.

The absence or presence of carnea could not be correlated with temperature in the field studies of June-July and September (Table VI). However, with more observations in the Ellerslie slough study, an apparent correlation with temperature was noted. H. carnea was first found when the temperature reached a level of 23.5°C while the disappearance was recorded at a temperature of 26.5°C (Figure 11). Better evidence of carnea temperature dependency was exhibited in a study of Lake Wabamun in the summer of 1963. At the power plant, the inlet canal is the home of H. canadensis as are other portions of the lake, ie: railway trestle, Fallis Point, etc. The outlet canal to the best of my knowledge is the only habitat of carnea in that lake. The pH remains the same (about 7.5) in both the inlet and outlet throughout the period studied (May to November). A chemical analysis of the water on May 15 and October 23, 1963 revealed no significant dif-

TABLE VIII

Data obtained from a chemical water analysis of Lake Wabamun.
Inlet and outlet of power (Calgary Power Co.) plant, May 15,
October 23, 1963. Analysis by the Provincial Analyst, Edmonton.

	PARTS PER MILLION			
	May 15		October 23	
	. Inlet	. Outlet	. Inlet	. Outlet
Total solids	244	244	314	312
Ignition loss	74	74	128	120
Hardness	115	115	135	120
Sulphates	6	6	35	46
Chlorides	nil	nil	nil	nil
Alkalinity	170	170	180	160
Nature of Alkalinity	Bicarbonate of lime, Mg. and Na. same		same	same
Nitrites	nil	nil	nil	nil
Nitrates	nil	nil	nil	nil
Iron	trace	trace	1.0	0.2

ference in the factors analyzed between these two areas (Table VIII). The only difference that can be shown between these two habitats is in the temperatures. The inlet canal follows the fluctuations of the lake while the outlet is maintained at a constant, year-round temperature of 21° to 22°C as a result of the operation of the power plant. Therefore temperature appears to be a determining factor in habitat selection of this species.

H. carnea becomes sexual more readily than any other species studied. Specimens brought to the laboratory usually become sexual within a few days. The rapidity with which the species becomes sexual would appear to offer advantages in the type of habitat described above. Changes in the composition of the water in these small shallow sloughs occurs rapidly as shown. Presumably at the first sign of deterioration of the water, sexuality occurs, resistant embryos are produced and the survival of the population is thus insured through the unfavourable period which follows. Therefore I would suggest that in this species gonad formation is induced by or accompanies an increase in temperature combined with an increase in pH. As described above this occurs in the sloughs inhabited by this species.

Hydra canadensis Rowan, 1930

Hydra pseudoligactis Hyman, 1931a, 1938, New Synonymy

Description

H. canadensis is a very large hydra, the column measuring up to 18 mm in length in the specimens collected



Male H. canadensis



Female H. canadensis

Figure 12. Sexual H. canadensis.

in this study. Fresh specimens usually have 5 to 7 tentacles, but the laboratory reared specimens may show up to 10. Tentacle length varies from 4 to 6 times the column length. Some specimens show a stalk but this feature is not as well marked as in other species, for example, H. oligactis.

The nematocysts sizes for my specimens are as indicated:

stenoteles	11.25 - 19.0 μ
holotrichs	10.5 - 14.0 μ
atrichs	8.25 - 12.5 μ
desmonemes	6.75 - 9.0 μ

Hydra canadensis is dioecious. The highly characteristic appearance of a stalk covered with numerous testes with nipples in the males and numerous ovaries in the females is illustrated (Figure 12). The spherical embryonic theca is very thin and delicate.

Rowan left no type specimens as far as I have been able to ascertain, but his description of this hydra accords so well in most respects with the large hydras found in the type localities (Beaverhill and Hastings Lake) that I am in no doubt concerning the identity of the latter. Rowan's description of this species differs from mine in tentacle number in that he states "In the adult the tentacles regularly number 6. No individual with more has been noted"; and further, concerning tentacle length, "the extremely delicate tentacles representing some five-sixths of the total length". These are not serious discrepancies since he maintained his hydras under natural conditions while mine were subject to an artificial medium and a rich diet of Artemia salina. The effect

of this has been noted above (see culture methods). Rowan also described "few and very low spines" associated with the theca, but I have not been able to see these in the material studied. This is however again a minor point and may only reflect a difference in the manner of viewing.

Hyman's description of H. pseudoligactis fits the Alberta specimens in all important respects. Specimens of H. pseudoligactis obtained from Dr. Helen Park also agree closely with the Alberta forms.

Hyman records a column length of 20 to 25 mm. for this species as compared to my measurement of 18 mm. However, the difference is not too important as length of column is a variable factor depending on the condition of the hydras and the culture medium in which they are observed. Tentacle lengths for Hyman's animals were 3 to 4 times column length as compared to 4 to 6 times column length in this study.

The size relations of the nematocysts of my specimens are in close agreement with Hyman's figures for H. pseudoligactis but, in absolute dimensions, the measurements are slightly higher.

	Alberta material	Hyman's
stenoteles	11.25 - 19.0 μ	10.0 - 17.0 μ
holotrichs	10.5 - 14.0 μ	10.0 - 12.0 μ
atrichs	8.25 - 12.5 μ	8.0 - 10.0 μ
desmonemes	6.75 - 9.0 μ	6.4 - 8.0 μ

The diagnostic features in this species which clearly separate it specifically from H. oligactis and aligns it with H. pseudoligactis are the external and internal appearance of the holo-



Figure 13. Holotrichous isorhiza of H. pseudoligactis from Hyman, (1931a).



Figure 14. A habitat of H. canadensis, Lake Wabamun.

trichous isorhizas (Figure 8). Hyman (1931a) states that "In pseudoligactis they are of elongated oval form with a transversely coiled thread. The thread makes about three transverse or oblique loops, often rather loose and irregular (my emphasis), and then becomes a tangled mass in the lower part of the nematocyst." A comparison of a canadensis holotrich (Figure 8) and that given by Hyman for pseudoligactis (Figure 13) admirably shows the similarity between the two. There is considerable variation between individuals from different localities in the external shape of the holotrich (Figure 8) but the internal characteristics are relatively constant. The other nematocyst types are shown in Figure 8.

The appearance of the animals in sexual condition described by Hyman is identical to that described by Rowan. Hyman also describes the embryonic theca as being thin and delicate. She does not mention the occurrence of spines on the theca as did Rowan. In this respect my specimens and Hyman's were identical.

Rowan's name H. canadensis has clear priority over the later H. pseudoligactis and so the species will be referred to as H. canadensis in this account. It may be worth mentioning that cultures of Hydra sp. sent in 1961 for class use from the General Biological Supply House, Chicago, and variously designated as H. littoralis, or H. oligactis were identified by Dr. Mackie as H. canadensis.

Distribution

This species has been reported throughout the northcentral U.S.A. by Hyman (1938). Forrest (1963) reports

its presence from New Jersey and Oregon and concludes with the statement "It appears that H. pseudoligactis may occur from coast to coast in the northern United States."

In Alberta, H. canadensis was captured in a number of lakes north of a line running roughly from Red Deer to Edmonton to Lloydminster (Figure 6). In the N.W.T. it was obtained less abundantly in localities to the west and southwest of Yellowknife (Figure 7).

Ecology

In Alberta this species was found in lakes of moderate size and depth (Figure 14), being conspicuously absent from both the small, shallow prairie sloughs and the large cool lakes of the mountain areas. An exception was the sample from the Sturgeon River (collected by A. Nimmo, Entom. department) which was found in the backwash of a flowing stream. In the N.W.T. specimens were found sparsely distributed in running water, in clear, clean, fairly deep (3 to 4 ft.) creeks. Hyman reports her specimens from the same area as H. oligactis (a deep water form). The bottom type was generally sandy in Alberta and ranged from humus to gravel in the N.W.T. In Alberta Typha sp. was often the substrate while narrow leafed Potamogeton sp. predominated as the favorite perch in the N.W.T.

The first canadensis specimens obtained in this study in the July 1962 survey of Alberta were asexual individuals from Twin Lakes and Crimson Lake. At this time the pH at both sites measured 7.5. Later specimens from other localities were also obtained from lakes which exhibited a

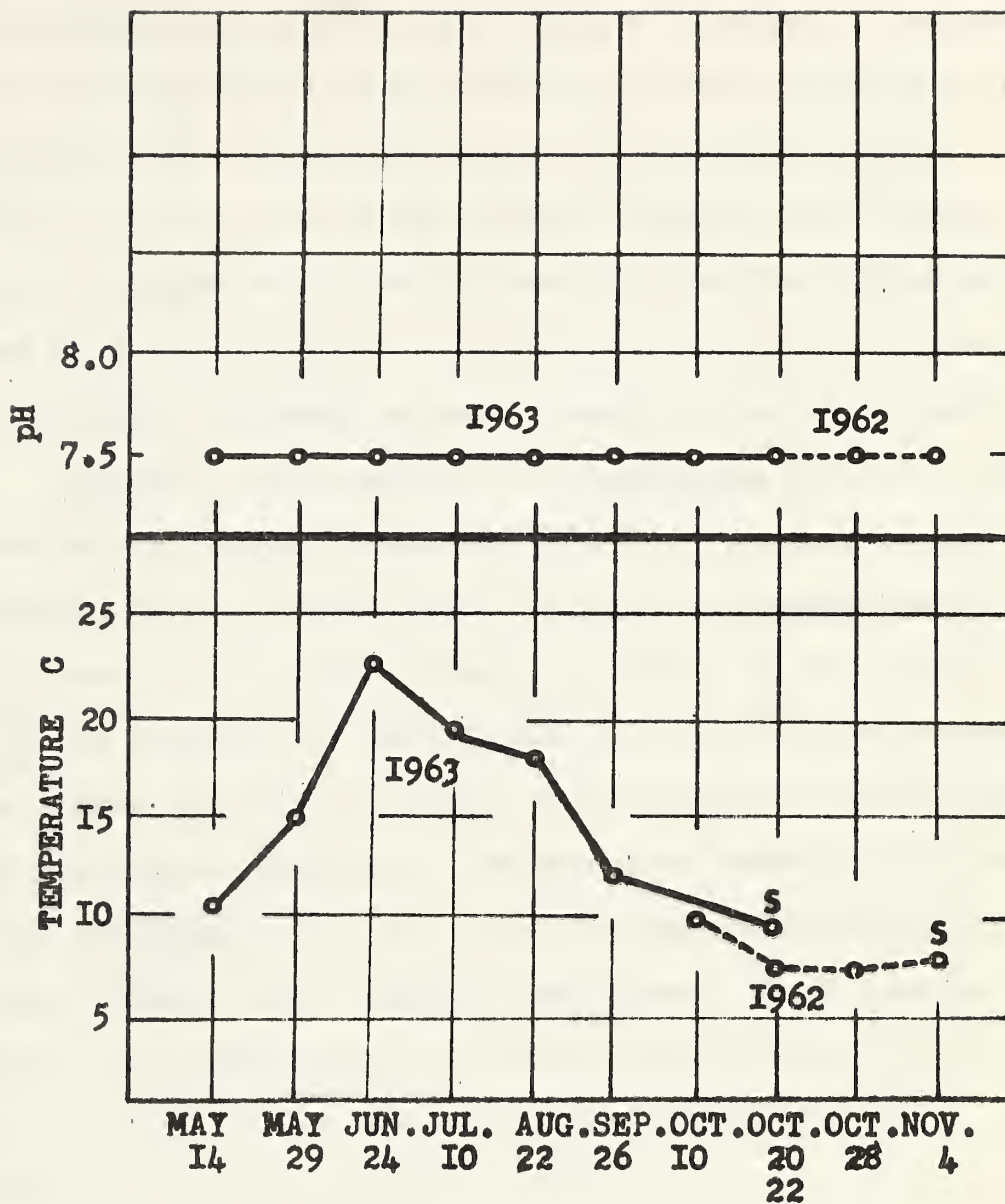


Figure 15. The seasonal variation in pH and temperature as related to H. canadensis existence, Lake Wabamun.

S = sexual hydras; asexual hydras found at all other collections.

slightly alkaline and relatively stable pH (around 7.5) irrespective of the time of year. Further study of the inlet canal of the power plant at Lake Wabamun throughout the summer of 1963 (from May 14 to October 20) revealed no deviation from a pH of 7.5 (within the error of the B.D.H. indicator solution) in the course of the season (Figure 15). The pH was still recorded at 7.5 on October 20 when the hydras were obtained in a sexual state. Therefore the pH did not seem to be the factor determining sexual maturity in this species.

General field sampling of canadensis did not reveal any correlation between temperature and the production of sex organs. Asexual hydras were obtained at temperatures varying from 10.5 to 22°C. However, a study of the inlet canal of the Calgary Co. power plant at Lake Wabamun throughout the summer of 1963 did reveal a correlation between temperature and sexual maturity. Temperatures taken at the level of the H. canadensis (ca. one foot) varied considerably in this study (Figure 15). Asexual individuals were obtained throughout the summer and it was not until October 20 at a temperature of 9.5° that sexual female individuals were recorded.

Rowan commented on "the marked periodicity exhibited in sexual reproduction" of this form. Gametogenesis generally ensues during the first week in October in the lakes near Edmonton. The males develop first, with the females developing a week or ten days later. Rowan stated that "breeding is practically over and the animals dying off by the end of the third week in October". Observations by Dr. G. O. Mackie

and Mrs. P. C. Adshead in the fall of 1961 and myself in the fall of 1962 and 1963 have been similar to those reported by Rowan. Therefore temperature seems to be a determining factor in sexual inducement in this species.

Hydra oligactis Pallas, 1766

Description

See Hyman (1930) for a more detailed description of this species.

This species has the most conspicuous differentiation of the column into a slender proximal stalk region and a stout body or stomach region of any species found in this study. Because of the presence of the stalk this species was formerly referred to the genus Pelmatohydra, but modern specialists (following Ewer, 1948) do not attach generic importance to the stalk or to the other characters used by Schulze (1917) in erecting that genus. Specimens obtained in Alberta measured up to 14 mm. in laboratory culture and thus were smaller than those reported by Hyman (up to 20 mm.). There are usually five to six tentacles, but the number is often higher in specimens reared in the laboratory. Tentacles were three to four times column length.

Values reported here for nematocyst length are slightly larger than those reported by Hyman, but the size relations between the four types are comparable.

stenoteles	11.5 - 16.5 μ
holotrichs	10.5 - 14.25 μ
atrichs	8.25- 10.5 μ
desmonemes	6.0 - 7.5 μ



Figure 16. A H. oligactis habitat, Barrier reservoir.



Figure 18. The H. littoralis habitat in Alberta,
Little Cameron Lake.

The sexes are separate in this species. Testes are numerous and without nipples while the theca is spherical and sparsely spinated.

Distribution

This is a very common species in many parts of the U.S.A., except probably in the southeastern states (Hyman, 1938). Ewer in his admirable summary of the Hydridae known prior to 1948 also lists records from Australia and Europe, including England.

In Alberta it was found in four locations: St. Mary's Reservoir, Beauvais Lake, Lake McGregor, and Barrier Reservoir on the Kananaskis River system. These localities are in the foothills area in the southwestern portion of the province (Figure 6).

Ecology

H. oligactis was found in certain large deep lakes and in Barrier Reservoir (Figure 16). The first specimens obtained were collected from depths between 10 ft. and 100 ft. in Barrier Reservoir by Denis Fillion in September, 1961. The hydras were found to be plentiful on the sandy bottom chiefly around a depth of 45 ft. The water temperature was about 10°C at this time. In 1962 asexual hydras were again obtained in the reservoir. In the other localities asexual hydras were obtained both in early July and in early September. The pH of the water remained near 7.5 during this period, while surrounding smaller bodies of water had undergone considerable increase in alkalinity as reported above for carnea

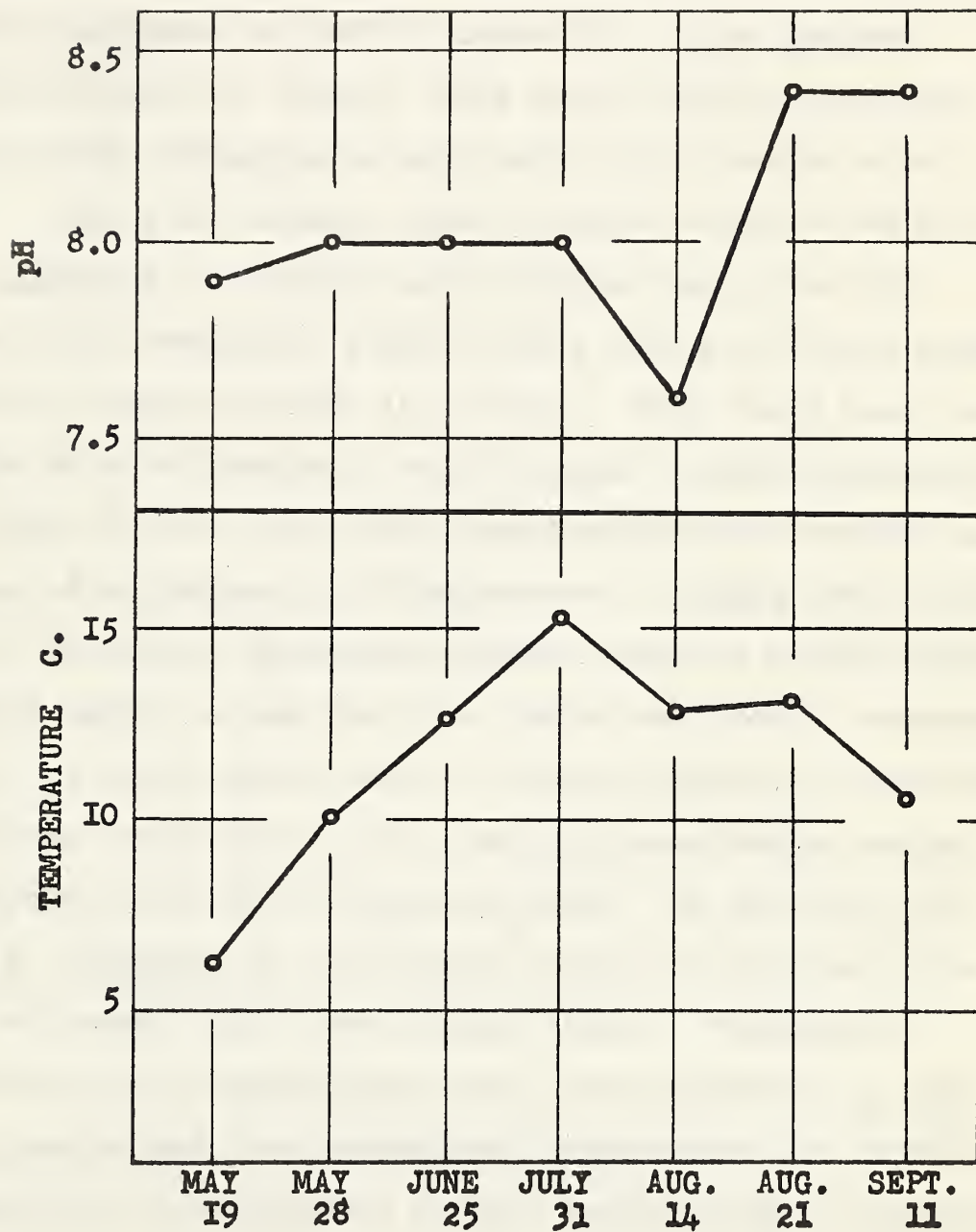


Figure 17. The seasonal variation in pH and temperature, Barrier Reservoir 1962.



1950 1951 1952 1953 1954 1955 1956

habitats. The temperature had decreased from 18° in July to 15°C in September. Temperature and pH records obtained from Fillion (personal communication) for Barrier Reservoir indicated little variation in temperature and pH for the period May 19 to September 11, 1962 (Figure 17). This species therefore appears to inhabit lakes which exhibit relatively low and steady temperatures with very little change in pH.

Welch and Loomis (1924) in their study of the ecology of H. oligactis in Douglas lake, Michigan found that the immense hydra population found in late spring and early summer ordinarily fades out later in the year. They found that over a period of seven seasons a daily maximum surface temperature above 72°F (22.2°C) and a daily minimum above 68°F (20°C) was accompanied by perceptible disappearance of Hydra from surface waters. It was not determined whether they had simply migrated to deeper waters or had died out. Welch and Loomis conducted a series of limnological tests in deeper regions and concluded that hydras could exist in this lake at great depths and at temperatures close to the freezing point. In fact they obtained H. oligactis in flourishing conditions from under the ice as did Hyman (1930) and Fillion (1963). Therefore it appears as if low temperatures have little effect on H. oligactis populations, but, rather, high temperatures are critical in causing the disappearance of this species from its habitat.

Hydra littoralis Hyman, 1931

Description

Identification of my specimens was based on the

description in Hyman (1931, 1931a, 1938).

This is a small species, the column not exceeding 12 mm in Alberta specimens. Hyman (1931a) has recorded lengths up to 15 mm. The stalk region is not differentiated from the column. The tentacles are 1.0 to 1.5 times the length of the column.

The lengths of the nematocysts are:

stenoteles	12.5 - 19.0 μ
holotrichs	10.0 - 12.5 μ
atrichs	8.25 - 9.0 μ
desmonemes	5.5 - 8.5 μ

The size relationships are as stated by Hyman except in certain specimens with disproportionally large desmonemes.

H. littoralis is dioecious. The males have large testes with stout nipples. The embryonic theca is spherical with long spines, as illustrated by Hyman (1938).

Distribution

H. littoralis has been reported from the following states: New York, New Jersey, Illinois, Pennsylvania and Oklahoma. Hyman (1938) suggests that it is probably widespread.

In Alberta, specimens were found only at Little Cameron Lake, Waterton Lakes National Park (Figure 18).

Ecology

Little Cameron Lake (altitude 5,400 ft.) is a wide portion of a swift creek running out of Cameron Lake. The temperature was 13°C when hydra were collected in early July,

1962. Specimens were again collected in early September 1962, at which time the temperature was 11.5°C. On neither occasion were the hydras sexually mature. This ecological situation (cool, running water) resembles that favoured by the species in other areas.

Hydra hymanae Hadley and Forrest, 1949

Description

This is a small hydra without an obvious stalk. The column length of fresh specimens did not exceed 4 mm, but specimens cultured in the laboratory extended to 10.0 mm. The tentacles are 0.5 - 1.0 times the column length. Freshly obtained specimens had five or six tentacles.

Nematocysts:

stenoteles	19.0 - 24.0 μ
holotrichs	10.0 - 12.5 μ
atrichs	8.25 - 10.75 μ
desmonemes	10.0 - 11.5 μ

The large size of the stenoteles and desmonemes give the cnidome of this species a large, rather distinctive appearance. The shape of the holotrich (Figure 8) is characteristic, with a well marked beak at the tip.

H. hymanae is hermaphroditic. Considerable variation in the shape of the testes is known and was noted. A nipple may or may not be present, and the general form may be compact or elongated. A maximum of three ovaries developing simultaneously have been observed on an individual hydra. The embryonic theca is plano-convex, and, on being



Figure 19. A typical H. hymanae habitat, Mile 14, Mackenzie highway. Hydras collected at foot of falls.



Figure 20. The habitat of C. hadleyi in Alberta, Lake Wabamun.

shed from the hydra, attaches by the flat side to the bottom of the culture dish. No spines were seen on the convex surface in the material studied, contrary to the findings of Hadley and Forrest (1949). However, these authors state that the spines show best in embryos formed in nature and may be absent in laboratory specimens. The only observations concerning the theca in my studies were made on laboratory specimens.

Distribution

The only previous records of this species are from various localities in New Jersey, U.S.A. In this study H. hymanae was found only in the N.W.T., where specimens were obtained from a total of seven bodies of water (Figure 7). In addition, a few specimens found at Mile 75 Mackenzie Highway, (Figure 7, no. 5) will be described as an atypical H. hymanae population.

Ecology

As with the specimens described by Hadley and Forrest, most of the specimens obtained in this study were found in running water (Figure 19) or in shallow lakes supplied by and close to running water. The specimens were not abundant, nor close to each other, but were sparsely and evenly distributed. The bottom type varied from hard clay to humus. The only two samples containing sexually mature individuals obtained in the field were collected from the only two bodies of water with a humus bottom. One sample was collected from

a slough containing lily pads (Nuphar variegatum), pH 7.0, temperature 16°C , on August 16, 1962 one mile south of Frank's Channel. The other sample was obtained on August 19, 1962 at Mile 35 (pH 7.5, temperature 15°C). The highest temperature recorded for this species was 17.5°C.

Hadley and Forrest (1949) recorded the appearance of sexually mature individuals around the first part of October with a return to asexuality by the first part of November.

Due to the paucity of specimens of hymanae, I was unable to determine the stimulus for gonad formation. The few specimens cultured in the laboratory were, however, prone to seemingly unexplainable, excessive, and debilitating gametogenesis.

Atypical H. hymanae

A hydra sample taken on August 18, 1962 from one location in the N.W.T. (Mile 75, no. 5, Figure 7) differed from typical H. hymanae. The body form and nematocyst shapes were in agreement with those described as typical of hymanae. The stenoteles, holotrichs and atrichs were in the range of hymanae, but the desmonemes were much smaller than the typical form. Their length was similar to that of the atrichs. No sexual individuals were observed.

The habitat of these specimens also differed from the characteristic H. hymanae habitat in being a shallow, dirty slough.

H. ?utahensis Hyman, 1931

A few specimens obtained from Lake Wabamun (at the inlet to the power plant) in 1961 were reported by Adshead (1961) as H. ?utahensis. A culture was not established at that time and a search of the area by myself in 1962 produced no additional specimens. However, this population closely resembled H. hymanae and the provisional identification was changed from H. ?utahensis to H. hymanae by Adshead, Mackie and Paetkau (in press) on the basis of the above description. To encourage further investigation into the problem of this species the main points of similarity to and differences from H. utahensis Hyman, 1931, and H. hymanae (my data) are summarized below.

The general characters of body shape and form and those of sex organs were similar in all three. The nematocysts sizes are as follows:

<u>H. utahensis</u>	Variant	<u>H. hymanae</u>
stenoteles 9 - 16 μ	13.5 - 24 μ	19 - 24 μ
holotrichs 8 - 9 μ	9 - 10.5 μ	10 - 12.5 μ
atrichs 6.4- 7.2 μ	6.75 - 7.5 μ	8.75- 10.75 μ
desmonemes 6 μ	6 - 7.5 μ	10 - 11.5 μ

The specimens are near hymanae in having large stenoteles. However the desmonemes are small, thereby resembling utahensis. An important taxonomic character, the shape of the holotrich, (although slightly more rotund) resembles that of hymanae in having a pronounced beak.

Genus Chlorohydra Schulze, 1917

The validity of the genus Chlorohydra is open to question. This genus is based solely on the presence of symbiotic zoochlorellae in the gastrodermis. However, green hydras have been deprived of their zoochlorellae by treatment with glycerine and successfully cultured without them (Forrest, 1959). In her study Forrest (1959) concludes with the statement "Although the genus Chlorohydra should probably lapse it is retained here pending further study". Due to the paucity of specimens in my study I am forced to take the same position.

Chlorohydra hadleyi Forrest, 1959

Only two green hydra have been reported in the world. Chlorohydra (Hydra) viridissima is the one commonly reported in the old or European literature. However, Forrest (1963) states that "all of the North American green hydras that I have examined have produced eventually the unique embryotheca of C. hadleyi". The presence of this double chambered embryonic theca readily distinguishes this species from viridissima.

Description

This is an extremely small, slender, green hydra measuring at most 5 mm in the specimens studied from Alberta. Forrest (1959) records column lengths up to 13 mm, with most individuals, however, measuring 7.0 - 7.5 mm in the asexual phase. The tentacles are shorter than the column and are held stiffly upwards. They number 6 to 9 in the material observed.

The nematocysts are smaller than those recorded for other hydras.

	Alberta material	Forrest
stenoteles	7.5 - 9.0 μ	6.75 - 12.5 μ
holotrichs	7.0 - 9.5 μ	8.25 - 9.75 μ
atrichs	5.0 - 5.75 μ	4.0 - 4.5 μ
desmonemes	5.0 - 5.75 μ	3.75 - 4.5 μ

The species is hermaphroditic. The testes are mammiform with nipples, and the ovaries usually develop above the budding zone. The embryonic theca is divided into a large distal and a shallow proximal chamber as figured by Forrest.

Distribution

Forrest, in her description of this hydra, recorded localities from New Jersey and Pennsylvania. This study adds one new location, Lake Wabamun, Alberta.

Ecology

This hydra was discovered (August 1962) several days after collection in a sample of aquatic plants taken from 4 to 6 feet of water (Figure 20). It was found in association with H. canadensis. Forrest remarks that, "these hydras have invariably been found in clear cool water in the vicinity of a spring, usually on the undersides of fallen leaves or clinging to the submerged stems of water plants, but sometimes attached to the lower parts of stones or to the undersides of sticks, boards, or pieces of broken glass". No spring has been discovered near the Lake Wabamun location.

DISCUSSION

Induction of sexual maturity

The disappearance of hydras from any locality is generally preceded by a phase of sexual reproduction which produces "over-wintering" eggs, thereby insuring the propagation of the species from season to season. Sexual maturity is not implicit in the biological cycle but is induced by external factors (Brien and Renier, 1949). Various external factors have been proposed as the causal factor stimulating or initiating sexual reproduction, with the most frequent being temperature and pH change.

To note only a few of the temperature experiments in order to see the trend, Stolte (1928) maintained a culture of asexual H. attenuata at 10°C and at 20°C and concluded from this that temperature had no effect on this species in regards to sexual inducement. However Schulze (1917) in his review states that workers as early as 1905-06 had shown that H. oligactis became sexual by lowering the temperature from 13° to 5°C, or by raising the temperature of H. vulgaris medium from 15° to 25°C. This has been verified by a number of workers including Hyman (1928). Itô (1952) in one of his series of studies on the reproduction of hydras (H. magnipapillata and H. paudicola) found the formation of gonads may be induced at a high rate in about one week by raising the temperature from 8° to 21°C.

There have been a few allusions to the ineffectiveness of pH in producing sexual maturity in hydras. Stolte

maintained cultures at pH 7.5 and 8.5 and stated that this proved that pH had no effect on sexual maturity. Threlkeld and Hall (1928) exposed H. oligactis and H. viridissima to a range of pH from 5.2 to 8.0 keeping the temperature at a constant 18 to 22°C. They found the pH optimum for both species to be 7.8 to 8.0 with animals dying off at an increasing rate with increasing hydrogen ion concentrations. Itô (1952) placed H. magnipapillata obtained from a medium with a pH of 7.2 in buffer medium of pH 6.4, 7.2 and 8.2. No sex organs were noted within twenty days.

Loomis (1957) reversibly induced gonad formation in H. littoralis by increasing the pCO₂ in the culture medium. In the same experiment he states "Since increasing levels of pCO₂ progressively decrease the pH of alkaline solutions, other experiments were conducted in which Hydra were cultured in solutions buffered with tris (hydroxymethyl) aminomethane. It was found that cultures of Hydra remained asexual regardless of pH when the animals are grown under uncrowded or aerated conditions". He does not state the pH tested. Park (1960) repeated and elaborated on Loomis's experiment again using H. littoralis. She concluded that "the percentage of hydras developing gonads when exposed to high pCO₂ may be lower than duplicate cultures exposed to CO₂ free air".

Keeping in mind that this is a different species our field study may enhance Dr. Park's argument. In the slough inhabited by H. carnea the pH obviously rose as shown by

Figure 11. It did not decrease as it should if increasing pCO_2 is the factor which induces sexual maturity and subsequent disappearance of this species from its habitat.

Therefore as indicated by the above study it appears that temperature and pH are the two main factors determining sexual maturity and the subsequent disappearance of a hydra from its habitat. However, it must be remembered that all of the species studied reacted to the effects of temperature and pH in a different manner. As a result I think that in this study, as in most biologically related studies, it is a mistake to look for one all-inclusive explanatory factor to explain the cause of sexual maturity in hydras.

The above study is interesting if one considers it in the light of evolution. Theoretically as in most other forms a primordial Hydra existed. This father-mother hydra has adapted quite well to many different ecological niches within the eons of time. In this study alone the adaptation to selection pressure can be seen. The study showed at least five ecologically separated and distinct species each with its own peculiar habitat and adaptation to it. This adaptation is specifically complete as has been indicated by the mentioned cross-fertilization experiments. In this study the presence of two or more distinct species inhabiting the same locality in certain zones of ecological overlap also revealed the distinctness of species. In these zones of overlap one can suggest that species separation began by physiologically induced (pH, temperature) reproductive isolation;

species became sexually mature at different times of the year. This separation has resulted in morphological separation as described for the species. In this study I could not see any correlation between the morphology of a species and the advantage this morphology would offer in a particular habitat. This problem warrants further study.

SUMMARY

The taxonomy, geographical distribution and ecology of species of the family Hydridae was studied in the province of Alberta and a portion of the N.W.T. during the spring and summer months of 1962 and 1963. Hydras were collected, identified, and if necessary cultured during this and the intervening winter period. Fresh specimens were used whenever possible. For supplementary studies, however, additional specimens from most localities were fixed in 70 per cent alcohol. An enzyme digestion method for isolation of nematocysts for viewing from fixed material is described.

A study of the effect of laboratory culturing on the nematocyst length (an important taxonomic character) was performed. It was found that the holotrichs of H. canadensis showed a highly significant increase in length after 70 days of laboratory culturing. The other nematocysts of H. canadensis did not show any difference. H. carnea nematocysts revealed no difference in lengths after a 50 day period of laboratory culturing.

A study of the natural seasonal variation of the nematocyst length in H. canadensis over a 134 day period showed a highly significant increase in holotrich, a significant increase in stenotele and a significant decrease in atrich lengths. A fifteen day period was not sufficient to show differences in the lengths of H. carnea nematocysts.

Stenotele and holotrich length of H. canadensis from two locations in southern Alberta and two locations in

the N.W.T. were compared. The results revealed a highly significant difference in holotrich length but no significant difference in stenotele length between the four localities. The holotrichs from the two Alberta localities did not differ, nor did the holotrichs from the two N.W.T. localities, but the Alberta forms were highly significantly larger than the forms obtained in the N.W.T. Specimens of H. carnea from two southern Alberta localities and one N.W.T. locality were compared. A comparison of the holotrichs showed a significant difference between either Alberta form and the N.W.T. form but no significant difference between the two Alberta forms. Stenoteles of the specimens from the two Alberta localities differed significantly but only one Alberta sample differed significantly from the more isolated N.W.T. sample.

The above tests revealed that nematocyst length varies intraspecifically, and this must be considered in the taxonomy of Hydridae. As a result, the conditions under which an animal is obtained and studied must be stated.

Two species, Hydra littoralis and Chlorohydra hadleyi, new to Alberta, were recorded. Three other species, H. canadensis, and H. carnea (reported by Rowan, 1930) and H. oligactis (reported by Adshead, 1961) were collected. The N.W.T. study produced three new records from that area, H. canadensis, H. carnea, and H. hymanae. A brief description of each species is given. H. pseudoligactis (Hyman, 1931) is shown to be a synonym of H. canadensis (Rowan, 1930).

Different species were found to inhabit different

geographical areas. H. carnea was found to be the common hydra of southeastern Alberta while H. canadensis was captured in a number of lakes in the northern portion of the province. H. oligactis was found in four locations in the foothills area in the southwest portion of the province. H. littoralis and Chlorohydra hadleyi were each obtained in one location, H. littoralis in the southeast corner of the province, C. hadleyi in Lake Wabamun.

In the N.W.T. H. carnea seemed to be limited to the area south of the Mackenzie river while H. canadensis and H. hymanae were found scattered throughout the area studied.

This geographical separation of the local species seemed to be determined by the ecological conditions found in the areas mentioned. H. carnea was found most frequently in the shallow muddy-bottomed sloughs or ponds typical of southeastern Alberta. H. canadensis was located in lakes of moderate size and depth while H. oligactis occupied large deep lakes. H. littoralis was found in a cool, clear, swift running creek while C. hadleyi was found in a moderate size lake.

In the N.W.T. H. carnea was again found in shallow sloughs or ponds while H. canadensis was found sparsely distributed in running water, in clear, clean, fairly deep creeks. H. hymanae was obtained in running water or in shallow lakes supplied by or close to running water.

The cause of the onset of sexual maturity with the subsequent disappearance of the animal from its habitat was

studied. It was concluded that a rise or high temperature combined with a rise or high pH caused sexual maturity in H. carnea. A decrease in temperature, or low temperature was a proposed cause in H. canadensis with a high temperature being a suggested cause for H. oligactis. Less conclusive evidence for the effect of temperature and pH on sexual maturity is reported for the other species studied.

Key to the described species, Family Hydridae, of Alberta and a portion of the Northwest Territories.

Figures stated are taken from living, expanded fresh material.

1. Symbiotic zoochlorellae present; tentacles shorter than column, up to 5 mm; two-chambered embryotheca, with extra, fluid-filled chamber between embryo and ovary of parent; rare in Alberta C. hadleyi
Forrest, 1959
- 1'. Symbiotic zoochlorellae absent 2
2. (1) Tentacles less than or equal to column length, column length up to 4 mm; holotrichous isorhiza broadly oval with prominent beak (Fig. 8); common in N.W.T. H. hymanae
Hadley and Forrest, 1949
- 2'. Tentacles greater than column length 3
3. (2) Holotrich kidney-shaped, with thread wound longitudinally throughout (Fig. 8); column up to 14 mm and often differentiated into body and stalk; a deep water (Alberta foothills) form. H. oligactis
Pallas, 1766
- 3'. Holotrich narrowly oval or cylindrical with irregular transverse or diagonal coils in the anterior part 4
4. (3) Coils (3 or 4) in anterior portion of holotrich loosely wound (Fig. 8 and 13);

column up to 18 mm; tentacles 4 to 6 times column length; sexes separate and embryotheca without spines; common in north-central Alberta and the N.W.T. H. canadensis

Rowan, 1930

4'. Coils (3 or 4) in anterior portion relatively tightly wound (Fig. 8) 5

5. (4) Sexes separate, testes unusually large with prominent nipples; column up to 12 mm; tentacles 1.0 to 1.5 times column length; habitat, running water; -rare in Alberta H. littoralis

Hyman, 1931

5'. Hermaphroditic but somewhat protandrous, testes small and mammiform; short theca spines on spherical theca (Fig. 9); obtained in standing water; common in southeastern Alberta and in the N.W.T... H. carnea

L. Agassiz, 1850

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APPENDIX I

The location of and ecological factors concerning hydras found in this study.

<u>Date</u>	<u>Location</u>	<u>pH</u>	<u>temp.C.</u>
<u>1962</u>	<u>In Alberta</u>		
	<u>H. carnea</u>		
June	Wabamun Lake Calgary Power Co. power plant outlet canal. Hydras obtained from bridge to lake.	7.3	22
"	Big Island Lake - along west shore	7	14
"	Beaverhill Lake - just south of south dam C. Kallal's farm pond.	7.5	18
"	Lake Newell - in narrows - east of Kinbrook Is. Provincial Park.	9	23
"	Spruce Coulee Lake - on south and east border of lake.	8	18
"	Reesor Lake - along shores of drifting islands.	8	18
"	Elkwater creek - at the beaver dams, in the area of Elkwater town campground.	7.3	19
"	Elkwater slough - 3 miles west of Elkwater on Hgh. #48, south side of highway	9.3	17
July	Crow Indian Lake - at dam straight south of Skiff.	7.3	15
"	St. Mary reservoir - in irrigation canal crossed by Highway #5.	7.2	15
"	Linnet Lake - seepage lake in Waterton town.	7.3	18

APPENDIX I (continued):

H. carneaIn Alberta

July	Frank Lake - along north-west shore.	8	16
"	Chestermere Lake - in north-east narrows.	8	18
Sept.	Linnet Lake - same.	7.5	14
"	Ribstone creek - at highway #13 crossing near Hughenden.	7.5	12.5

1963

June	Slough - 4 miles directly south of Kitscoty east side of road.	7.5	22
"	Pond - 11 miles south of the N. Sask. River on highway #41.	7.6	23
July	Devils Lake - on all vegetation.	--	--

In the Northwest Territories1962

August	Mile 44.7 - one half mile north of highway.	7.5	19
"	Mile 5.5 - barrow pit (dugout) north side of road.	7.6	19.5
"	Mile 33 -	7.3	16.5
"	Mile 66 - one mile west of D.P.W. road.	7.5	18

H. canadensis1962 In Alberta

July	Twin Lakes - along east shore.	7.5	19
"	Crimson Lake - in the center of the north- east narrows, on <u>Typha</u> sp.	7.8	22
"	Hastings Lake - general.	7.5	22

APPENDIX I (Continued):

H. canadensis1962 In Alberta

July	Wabamun Lake - inlet canal of the power plant.	7.5	20
Sept.	Moose Lake - at Vezeau beach.	8.5	11
"	Cache Lake - at outlet crossing highway #28.	7.2	10.5

1963

July	Upper Mann Lake - along west shore.	7.5	21
August	Talbot Lake - along north shore.	--	--
"	Chip Lake - along south east shore.	8.5	16
October	Sturgeon Lake - along south shore.	--	--

1962 In the Northwest Territories

August	Mile 10.3 -	7.2	18
"	Mile 23 -	7	18
"	Mile - 6 miles west of Yellowknife.	6.5	18
"	25 miles west of Yellowknife.	7	18.5
"	5 miles south of Stagg river.	6.5	19.5

H. oligactis1962 In Alberta

July	St. Mary reservoir - same.	7.2	15
"	Beauvais Lake - in the east narrows.	7.5	18
"	Lake McGregor - in the north end, in not shallower than six feet of water.	7.5	16
	Barrier Reservoir -		
Sept.	St. Mary reservoir - same.	7.5	16

APPENDIX I (Continued):

H. littoralis1962

July	Little Cameron Lake.	7	13
Sept.	Little Cameron Lake.	7	11.5

H. hymanae1962 In the Northwest Territories

August	Mile 50.2.	7.3	17.5
"	Mile 66 - 10 miles west on the D.P.W. road.	7.3	16
"	Stagg river - at campground, east side of road.	7	17.5
"	Beside highway, one mile south of Frank's channel.	6.8	16
"	Mile 39 -	7.5	15
"	Mile 35 -	7.5	15

Chlorohydra hadleyi1962

August	Wabamun pier.	--	--
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APPENDIX II

Ecological factors concerning locations where hydras were not found in this study.

<u>Date</u>	<u>Location and Ecology</u>	<u>pH</u>	<u>Temp.C.</u>
<u>1962</u>	<u>In Alberta</u>		
June	Elkwater Lake - investigated completely.	7.5	18
"	Stirling Lake - investigated completely.	10.5	17
"	St. Mary reservoir - in region of dam.	--	--
"	St. Mary river -	--	--
July	Slough one mile west of Mountain View.	7.8	17.5
"	Waterton river - in the region of the Park gates.	7	15
"	Pincher creek - where highway #2 crosses it.	8	20
"	Oldman river - where highway #2 crosses it.	7.5	19
"	Mud Lake - complete.	9.8	18.5
"	Willow creek - at crossing of highway #2.	8.3	21
"	Irrigation canal at Bassano	8	18
"	Eagle Lake - complete.	11	21
"	Kanakaskis river - where highway #1 crosses it.	7.5	12.5
"	Seebe Dam - region.	--	--
"	Hart Lake - complete.	8.5	18
"	Bowfort Lake - complete.	8	15
"	Randone Lake - at the junction of the Ghost and Bow River.	--	--

APPENDIX II (Continued):

July	Little Red Deer - at crossing of Highway #27.	7.5	18
"	James river - at the James River Bridge.	7.3	20
"	Slough at Bowden -	10	23

In the Northwest Territories

August	Twin Lakes -	7	21
"	Kakisa Lake - explored the north shore.	7.5	21.5
"	Slough at junction to Lady Evelyn falls.	7.5	19
"	Mile 51.5 - west of Enterprise, slough.	7.3	17
"	Mile 42.5 - west of Enterprise, shallow slough.	7.4	19
"	Mile 14 - west of Enterprise, clear water creek.	7.	17.5
"	Mile 37 - west of Enterprise, average slough.	7.4	20
"	40.5 - west of Enterprise, small slough.	7.5	19.5
"	Mile 60 - west of Enterprise, small creek.	7	15
"	Mile 61 - west of Enterprise, shallow slough.	7.4	19
"	Mile 62 - west of Enterprise, large slough.	7.4	19
"	Mile 64 - west of Enterprise, small humus slough.	8	21
"	Mile 70.5 - west of Enterprise, small creek.	7.3	18

APPENDIX II (Continued);

August	Mile 66 - 2 miles west on D.P.W. road shallow muskeg slough.	7.5	17.5
"	Mile 66 - 9 miles west on D.P.W. road - lake.	7.5	19.5
"	Mile 66 - 16 miles west on D.P.W. road - lake.	7.5	19
"	Long Lake - large lake, sampled along east side.	7	17.5
"	17 miles west of Yellowknife on highway a shallow "lily pond" lake.	7	20
"	23 miles west of Yellowknife - "lily pond".	--	--
"	43 miles west of Yellowknife - "lily pond".	7.3	20
"	1/2 mile south of Stagg river - small creek.	6.5	9.5
"	One mile south of Stagg river - "lily pond".	7	14
"	1/2 mile east of Rae - large creek.	7	17
"	5 miles south of Frank's canal - a river, near mouth into Great Slave Lake.	7	16
"	Lake - origin of Mosquito creek, creek also checked.	7.5	17
"	Bluefish river -	7.6	15
"	Mile 215 - a dugout.	7.5	15.5
"	Mile 120 - dugout and creek.	7.5	14

APPENDIX II (Continued):

In Alberta

Sept.	Steveville Dinosaur Park - sampled all available water.	--	--
"	6 miles west of Badlands - on road to Patricia small slough fed by irrigation ditch.	10	17
"	Lake Newell - narrows east of Kinbrook Is. Prov. Park.	9	17.5
"	Spruce Coulee Lake - as in June.	8	8
"	Slough 3 miles west of Elkwater, Highway #48 as in spring.	10	10
"	Crow Indian reservoir - as in July.	11	17.5
"	Frank Lake - as in July.	--	--
"	Gull Lake - south portion of lake.	9.5	12.5
"	Cap Eyre - small slough.	9.5	13
"	One mile east of Alex, north side of road - slough.	9	14.5
"	Rochon Sands area of Buffalo Lake.	10	14
"	Gooseberry Lake -	11	12
"	Slough directly west of Auburndale community center.	9.5	12
"	Kenilworth Lake.	11	11
"	Cameron Lake.	9.5	9
"	Raft Lake.	7.5	10
"	Creek joining two portions of Raft Lake.	9.5	9.5
"	Landon Lake.	11	9

APPENDIX II (Continued):

Sept.	Bonnyville Lake.	7.5	11
"	Lake between Saddle and Cache Lake.	7.5	8
"	Vincent Lake - sampled on south side of lake.	7.5	11.5
"	Smoky Lake -	7.5	11.5
<u>1963</u>			
June	Rochon Sands - as in Sept.	9.6	18
"	4 miles south of Gadsby on road to Leo - slough.	10	20
"	Shooting Lake.	7.5	20
"	Gooseberry Lake - as in Sept.	12	20
July	Kenilworth Lake - as in Sept.	9.5	23
"	Vermilion Prov. Park Lake.	7.5	20
"	St. Paul - lake one mile south.	11	24
August	Mildred Lake.	10	17
"	Edna Lake.	7.2	15

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